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The Maillard Reaction

CHEMISTRY, BIOCHEMISTRY AND IMPLICATIONS

HARRY NURSTEN

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Chemistry, Biochemistry and Implications

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Harry Nursten
The University of Reading, Reading, UK

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advancing the chemical sciences

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Preface

The Maillard reaction was given its name in honour of Louis-Camille Maillard, who first described it in 1912. It is the reaction between an amino compound, often an amino acid, peptide, or protein, and a carbonyl compound, usually a reducing sugar, such as glucose, fructose, or lactose. Since such compounds are present in virtually every cell, the ramifications of the Maillard reaction are almost boundless and understanding it is of fundamental importance to food science and to the functioning of living cells. However, it has significance also in many other areas, such as soil science, aspects of textiles, and pharmaceuticals.

Its importance is such that well attended international symposia have been devoted to it on a regular four-yearly basis since 1979. Each of these symposia has led to a book of collected papers and poster presentations. There have also been many more local symposia, particularly in Japan, but, up to the present, there is no single-author volume, summarising the Maillard reaction and inter-relating its many facets. This is what is attempted here, with the intention of ensuring that the advantages of more disciplined and uniform treatment outweigh the inevitable shortcomings.

Personally, I became aware of the Maillard reaction as early as 1947, as part of the final year course in Colour Chemistry given by E.J. Cross at the University of Leeds and my interest was reinforced by the lectures of Marc Karel at the Massachusetts Institute of Technology in 1961 on its role in food science and technology. The latest chromatographic techniques were involved at each stage of my other research work and it seemed that the separation capabilities had become such as to be able to achieve progress with unravelling the almost prohibitively complex Maillard systems. Research into aspects of the reaction therefore followed, largely through collaboration with a series of postgraduate students and postgraduate fellows, encompassing both aroma compounds and coloured compounds originating in the reaction. I am greatly indebted to each of these colleagues and to the many fellow scientists, who became my friends, over the years all over the world.

Contents

Abbreviations

ABAP	AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride
aBTS	2,2′-Azinobis-3-ethylthiazoline-6-sulfonate
aDIBA	α,α'-Azodiisobutyramidine dihydrochloride
AGE	advanced (intermediate) glycation endproduct
ALE	advanced lipoxidation endproduct
AMP	AMRP, advanced Maillard reaction product
AOXP	antioxidative potential
AP	amadori product
AU	absorbance unit
a_w	water activity
BHA	butylated hydroxyanisole
BHT	butylated hydroxytoluene
BSA	bovine serum albumin
CAV	colour activity value
CML	N^{ε}-carboxymethyllysine
CP-MAS	cross-polarization-magic angle spinning
cTDA	comparative taste dilution analysis
CCR	cytochrome c oxidoreductase
DFG	*N*-(1-deoxyfructos-1-yl)glycine
3-DG	3-deoxyglucosone, 3-deoxyglucosulose
DH	deoxyhexosone, deoxyhexosulose
DMPD	*N*,*N*-dimethyl-*p*-phenylenediamine
DPC	degree of phosphate catalysis
dPPH	α,α-Diphenyl-β-picrylhydrazyl
DTPA	diethylenetriaminepentaacetic acid
EAGLE	either an advanced (intermediate) glycation or an advanced lipoxidation endproduct
EDTA	ethylenediaminetetraacetic acid
ESI	electrospray ionisation
FAB	fast atom bombardment
FL	fructosyllysine
FRAP	ferric reducing ability of plasma
GIM	glucosylisomaltol
GST	glutathione S-transferase
HAA	heterocyclic aromatic amines
HDMF	furaneol™, 4-hydroxy-2,5-dimethylfuran-3-one

HEPES	4-(2-hydroxyethyl)piperazine-1-ethylsulfonic acid
HMF	hydroxymethylfurfural
HNE	4-hydroxynonenal
IARC	International Agency for Research on Cancer
LPP	limit-peptide pigment
MALDI-TOF	matrix-assisted laser desorption/ionisation time-of-flight
MDA	malondialdehyde
ORAC	oxygen radical antioxidant capacity
PBN	phenyl *N*-t-butylnitrone, *N*-t-butyl-α-phenylnitrone
PIPES	piperazine-1,4-bis(2-ethanesulfonic acid)
SMP	skim milk powder
STZ	streptozotocin
TEAC	trolox equivalent antioxidant capacity
TBA	thiobarbituric acid
TES	*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid
TFA	trifluoroacetic acid
TPTZ	2,4,6-tris(2-pyridyl)-s-triazine
TRAP	total radical trapping activity of plasma
UHT	ultra-high temperature
VCEAC	vitamin C equivalent antioxidant capacity

CHAPTER 1

Introduction

1 Categorisation

There are two main types of mechanism by which browning of food occurs, depending on whether the process is mediated by enzymes or not. The division is not precise and, in a specific case, it is usually difficult to rule out one or the other mechanism, unless conditions are such, for instance, during heat processing, that enzymes would have been inactivated.

Under such conditions, only nonenzymic browning can occur. Nonenzymic browning itself is subdivided roughly (again because there is an overlap) into three types of reactions.

The first, called the Maillard reaction,[1] occurs between a carbonyl compound, which here is usually a reducing sugar, and an amine, which here is usually an amino acid, a peptide, or a protein. The second is caramelisation, a reaction where the sugars react on their own, but normally requires more drastic conditions. (Some discuss this under the heading of 'active' aldehydes.) The third is ascorbic acid oxidation. The last, although it need not involve any enzyme at all, is nearest to enzymic browning, since it often does involve ascorbic acid oxidase, which, however, does not affect the phenols, which are the normal substrate in enzymic browning, but may involve other enzymes, *e.g.*, laccase or peroxidase.

Here, much attention will be given to the Maillard reaction, since one can consider caramelisation and ascorbic acid oxidation as special cases of it. Also, the Maillard reaction is the one of physiological significance.

2 Historical: Louis-Camille Maillard

Maillard (1878–1936) qualified in medicine from the University of Nancy in 1903, where he joined the Chemical Division of the School of Medicine.[2] In 1914, he became the head of a biological group in the Chemical Laboratory, University of Paris, and, in 1919, he was appointed as the Professor of Biological and Medical Chemistry at the University of Algiers.

Maillard was interested in Emil Fischer's synthesis of peptides, which he thought, correctly as it turned out, could be achieved under milder conditions by the use of glycerol.[3] This logically led on to the use of sugars as another type of polyhydroxy compound to bring about the formation of peptides and to the discovery that

reducing sugars showed extra reactivity.[1] He published seven more papers on the sugar–amino acid reaction (see ref. 2). Further light has recently been shone on Maillard's career.[639]

Robert Ling (1861–1937), Lecturer in Brewing and Malting at the Sir John Cass Institute, London, had earlier noted that kilning led to amino compounds being produced from proteins, and that these reacted at 120–140 °C with sugars, such as glucose and maltose, produced simultaneously, giving what he thought were probably glucosamine-like compounds.[4]

3 The Maillard Reaction

The Maillard reaction is incredibly complex. For instance, a simple example such as the reaction of glucose with ammonia gives evidence, using simple methods, of the formation of more than 15 compounds and the reaction of glucose with glycine gives more than 24. Using HPLC and TLC on solvent-soluble material only [0.1% (w/w) of reactants], about 100 components are detectable as reaction products of xylose and glycine.[5]

In order to understand something so complex, it is necessary to draw up a simplified scheme of the reactions involved. This has been done most successfully by Hodge[6] (see Scheme 1.1). The discussion here is based on this.

Hodge subdivides the Maillard reaction as follows:

I *Initial stage:* products colourless, without absorption in the ultraviolet (about 280 nm).
  Reaction A: Sugar–amine condensation
  Reaction B: Amadori rearrangement

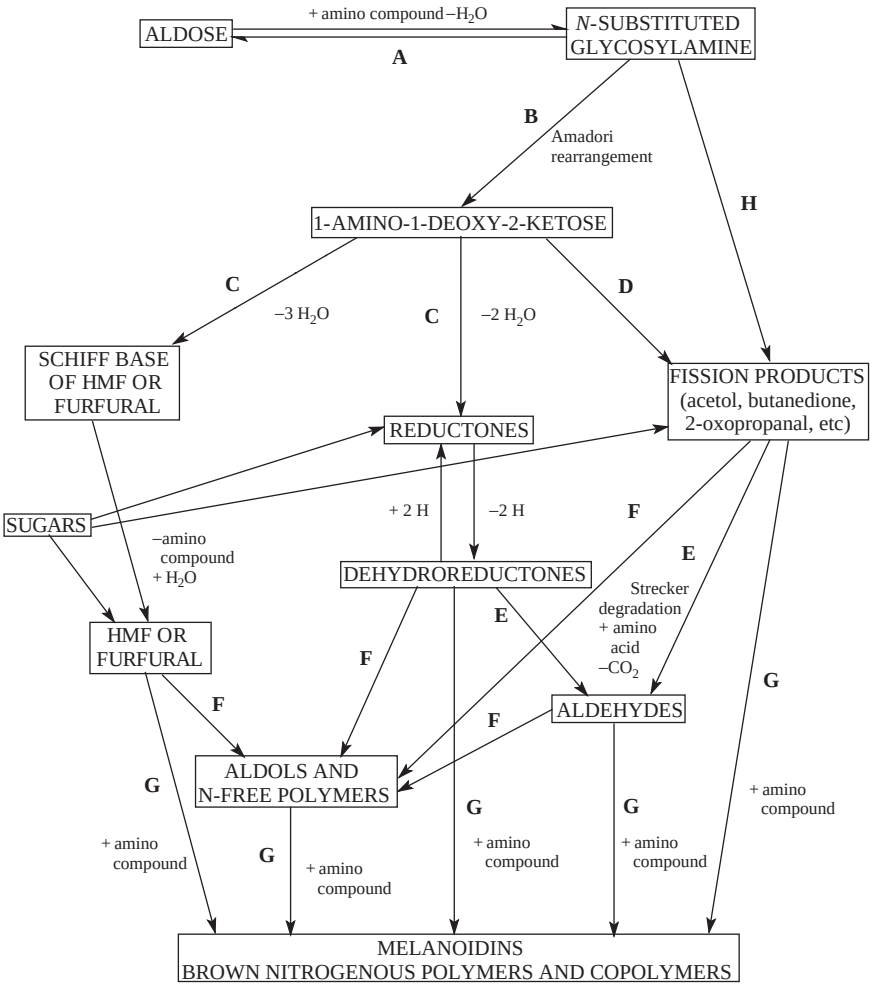
II *Intermediate stage:* products colourless or yellow, with strong absorption in the ultraviolet.
  Reaction C: Sugar dehydration
  Reaction D: Sugar fragmentation
  Reaction E: Amino acid degradation (Strecker degradation)

III *Final stage:* products highly coloured.
  Reaction F: Aldol condensation
  Reaction G: Aldehyde–amine condensation and formation of heterocyclic nitrogen compounds

It is worth noting that Mauron[7] calls the three stages Early, Advanced, and Final Maillard reactions, respectively. The way these reactions fit together is outlined in Scheme 1.1. The final products of nonenzymic browning are called melanoidins to distinguish them from the melanins produced by enzymic browning. Theoretically, the distinction is clear; however, in practice, it is very difficult to classify the dark-brown products formed in foods, since they tend to be very complex mixtures and are chemically relatively intractable.

Reaction H has been inserted into Scheme 1.1. It represents the much more recently discovered free-radical breakdown of Maillard intermediates (see Chapter 2).

Oxygen plays an essential role in enzymic browning, but is not essential for nonenzymic browning. It may help in fact, for example, in the formation of



Scheme 1.1 *Nonenzymic browning* (*based on Hodge*[6])

reductones, such as dehydroascorbic acid, but it may also hinder the progress of the reaction, for example, in oxidising 2-oxopropanal to 2-oxopropanoic acid.

Table 1.1 lists 12 symptoms of nonenzymic browning and shows how these develop in relation to the three stages of it. Note in particular that, as far as browning itself is concerned and also off-flavour production, there is an induction period.

The Maillard reaction is exceptionally widespread. It occurs virtually ubiquitously in foodstuffs, particularly during processing at elevated temperatures (roasting, baking, extruding) or during storage for prolonged periods. It is important in the manufacture of reaction flavours, coffee, and chocolate. It occurs also in textiles. It plays a complex role in humic substances in the soil and the sea. The changes it brings about in food have both nutritional and toxicological effects. It has important medical implications, since it occurs in the body wherever there is contact between

Table 1.1 *Nonenzymic browning*

No.	*Symptom*	*Stage* *Initial*	*Intermediate*	*Final*
1.	Production of colour of discoloration	−	+	+++
2.	Production of flavour or off-flavour	−	+	++
3.	Production of water	+	+	+
4.	Production of carbon dioxide	?	+	?
5.	Lowering of pH	?	?	?
6.	Increasing reducing power (antioxidant activity)	+	+	+
7.	Decreasing solubility	−	−	+
8.	Loss of vitamin C activity	+	−	−
9.	Loss of biological value of protein	+	+	+
10.	Chelation of metals	−	?	+
11.	Development of toxicity	−	?	?
12.	Production of fluorescence	−	+	+

After Karel[8] and Nursten.[9]

amino compounds and reducing sugars, particularly for prolonged periods (*e.g.*, aging, cataract, diabetes, and dialysis fluid).

The reactions contributing to the Maillard reaction will be discussed further individually in the next chapter.

4 The Literature

The most important set of volumes on the Maillard reaction are the books recording the papers presented at the international Maillard symposia, held about every four years, since the first, in Uddevalla, Sweden, in 1979.[A–G] Also important are the books resulting from the European cooperation in the field of science and technical research, COST Action 919.[10–13] There are many other important books that deal with aspects of the Maillard reaction, and reference will be made to these, when papers in them are quoted. Three books specifically on the Maillard reaction are those by Baynes and Monnier,[14] Ikan,[15] and Fayle and Gerrard.[16] Important review articles are those by Reynolds,[17,18] Namiki,[19] and Ledl and Schleicher.[20]

CHAPTER 2

The Chemistry of Nonenzymic Browning

Hodge's scheme was outlined in Chapter 1, where it was shown to be composed of eight types of reactions, A to H. These will now be considered further in turn:

1 Reaction A: Sugar–Amine Condensation

This reaction can be formulated as in Scheme 2.1.

It should be noted that each of the steps in Scheme 2.1 is reversible. The amine can be a protein, and it has been shown that insulin will react with glucose at a significant rate even at room temperature.

There is no fundamental reason why the glycosylamine should not act as the amine for a further molecule of aldose, thus giving a diglycosylamine.

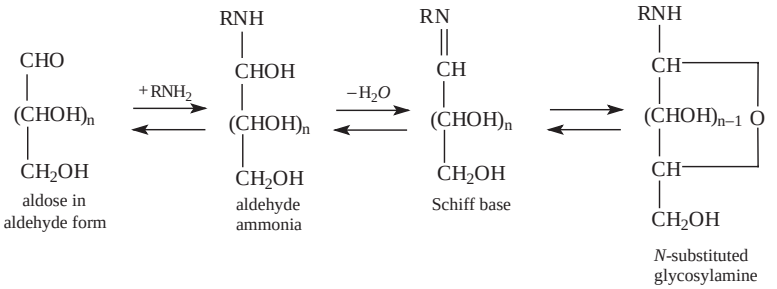
N-Substituted glycosylamines on mild heating give fluorescent nitrogenous compounds, which can rapidly react with glycine to give melanoidins. It seems therefore that a reaction of type G can, under certain circumstances, short-circuit Reaction B, *etc*. (*cf*. Reaction H).

Lysine locked up as ε-glycosylamine appears to be nutritionally available.[7]

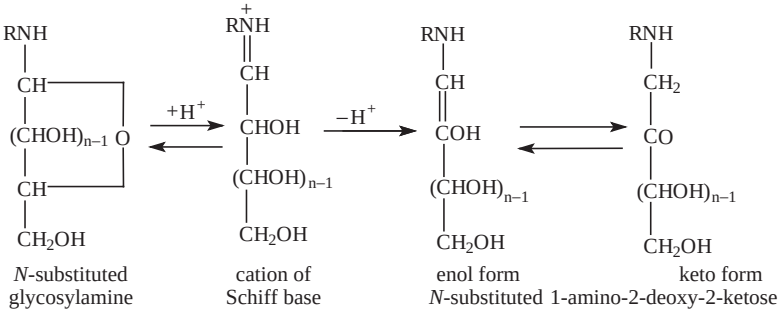
2 Reaction B: Amadori Rearrangement

This reaction, which is thought to be acid-catalysed, can be depicted as in Scheme 2.2.

It is important to note that overall the Amadori rearrangement is not reversible (but see below). The reaction takes place spontaneously even at 25 °C. The mechanism depicted by Scheme 2.2 is supported by the fact that, if the hydroxy group at C-2 is blocked by, *e.g*., a methyl group, rearrangement becomes impossible. Further support comes from the long-established facts that 11 fructosylamino acids and two difructosylamino acids have been found in stored, freeze-dried apricots and peaches and some have also been detected in dehydrated carrots, cabbage, spray-dried tomato powder, soy sauce, tea (derived from glutamic acid, theanine), beet molasses, liquorice, sterilized and condensed milk, dried skim milk and whey, infant food, roasted meat, cartilage collagen, and calf and hog liver extracts.



Scheme 2.1 *Sugar–amine condensation to form* N*-substituted glycosylamine*



Scheme 2.2 *Amadori rearrangement leading to the Amadori compound, the* N*-substituted* 1*-amino*-2*-deoxy*-2*-ketose*

More recently, Eichner *et al*.[22] have determined up to 12 Amadori compounds in tomato powder and in dried bell and red peppers, asparagus, cauliflower, carrot, and celery, accounting for up to more than 9% dry weight (tomato powder). Fructosylpyrrolidonecarboxylic acid was among the Amadori compounds detected. It can be formed from the glutamic acid or glutamine compound, its production indicating more extensive exposure to heat. In the case of malts, dark malts contain more Amadori compounds than light ones, but none survive into very dark malts, exposed to 200 °C. Eichner *et al*. found eight Amadori compounds in cocoa beans (almost 100 mg total per 100 g fat-free) before roasting, implying their formation under the relatively mild conditions of fermentation and drying of the beans.

Eichner *et al*.[23] also showed that fructosylglycine decomposes progressively at 90 °C in a citrate buffer (pH 3.0), 3-deoxyglucosone (3-DG) reaching a maximum concentration in about 15 h, whereas hydroxymethylfurfural (HMF) increases continuously, but more slowly after 24 h. The difference between the glycine liberated and the fructosylglycine lost widens progressively, implying that glycine is involved in further reactions. Whereas the Amadori compound is decomposed to $>90\%$ in 3 days at pH 3, at pH 7, it only takes 8 h. At pH 7, HMF is not detected and 3-DG is only formed in small amounts, but more browning is evident.

Fresh garlic contains no N^{α}-fructosylarginine; however, in the manufacture of aged garlic extract (over 10 months or so), it increases progressively,[24] providing potent

antioxidant activity. The two pairs of tetrahydro-β-carbolines, isomeric at position 1, the 1-methyl-3-carboxy and the 1-methyl-1,3-dicarboxy, are also not present in fresh garlic, but increase progressively in aged garlic extract.[25] They too are potent antioxidants.

The analysis for Amadori compounds has been reviewed by Yaylayan and Huyghues-Despointes.[26] Recently, high-performance ion-exchange chromatography coupled with pulsed amperometric detection has been advocated as a method that gives excellent resolution, yet is highly selective and sensitive for the detection of sugar without derivatisation at picomole levels with minimal clean-up.[21] For glucose and for fructosylglycine, a linear response was obtained up to 100 nmol mL^{-1}, with a detection limit of about 200 pmol mL^{-1}. Good separation of Amadori compounds from Pro, Gly, Val, Ile, and Met was demonstrated.

More recently, acid hydrolysates have been analysed by HPLC.[27] Furosine (see below) was the main product observed for dried figs and apricots, whereas furosine and the γ-aminobutyric acid derivative, in about equal amounts, were the main products for prunes and dates. 2-Furoylmethyl-γ-aminobutyric acid and -arginine were the most abundant products observed for commercial raisins, ranging from about 10 to 75 mg per 100 g of sample each. Most of the Amadori compounds present in raisins seem to have been formed during storage rather than processing.

Using their method, Blank *et al*.[21] examined the stability of fructosylglycine in solutions at different pH values. At 100 mM, 90 °C, 7 h, in water at pH 5, 6, 7, and 8, about 70, 31, 3, and 0% remained undegraded, whereas in 0.1 M phosphate the corresponding values were about 35, 0, 0, and 0%. After 1 h, the values had been about 98, 90, 60, and 34% and 95, 63, 30, and 24%, respectively. The degradation was clearly favoured both by higher pH and by the presence of phosphate.

Compared with the *N*-substituted glycosylamines, the 1-amino-l-deoxy-2-ketoses are more stable to moist acid atmospheres, but are still heat-labile and decompose rapidly in mild alkali. They exert greater reducing power, although less than reductones. They brown more easily with amino acids. Acid hydrolysis gives much compared with little HMF, but *no* hexose is recovered, in keeping with the reaction's being irreversible (however, see below).

Ketoses undergo a similar series of reactions, leading to 2-amino-2-deoxyaldoses (Heyns rearrangement). However, browning reactions of fructose differ from those of glucose, *e.g*., loss of amino acid or of free amino groups (casein) is much lower.[28]

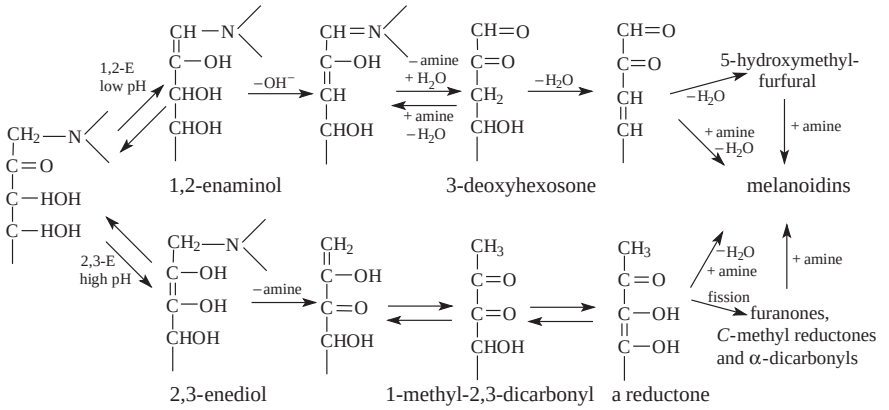
Thermal degradation of Amadori compounds, *e.g*., at 250 °C for 15 min, has been investigated by Birch *et al*.[29] They used glucose, ^{14}C-labelled at C-l, C-2, or C-6, and β-alanine. C-2 was retained, but most of C-1 and/or C-6 was lost, depending on the product.

The pathways by which Amadori compounds lead to other products are given in more detail in Scheme 2.3.

The following should be noted:

1. The relatively stable Amadori compounds can react essentially by two routes:
   - 1,2-enolisation via 3-deoxy-1,2-dicarbonyls,
   - 2,3-enolisation via 1-deoxy-2,3-dicarbonyls,

   the choice being effected mainly through pH, a low pH favouring 1,2-enolisation and *vice versa*.



Scheme 2.3 *Maillard reactions: the two major pathways from Amadori compounds to melanoidins* (*based on Hodge*[273])

2. Certain key steps appear to be irreversible (but see below):
   - *N*-substituted glycosylamine → 1,2-enaminol (Scheme 2.2),
   - 1,2-enaminol → 3-deoxy-1,2-dicarbonyl,
   - loss of amine from 2,3-enediol.
3. The relation between the two competing routes within the 1,2-enolisation pathway (pyrraline:β-dicarbonyl) has been found to be 70 : 30 by means of mass spectrometry applied to [1-^{13}C and 5-^{13}C]pentose systems.[30]

Lysine locked up in ε-Amadori compounds becomes nutritionally not available; this poses an analytical problem.

2.1 Determination of Nutritionally Blocked Lysine

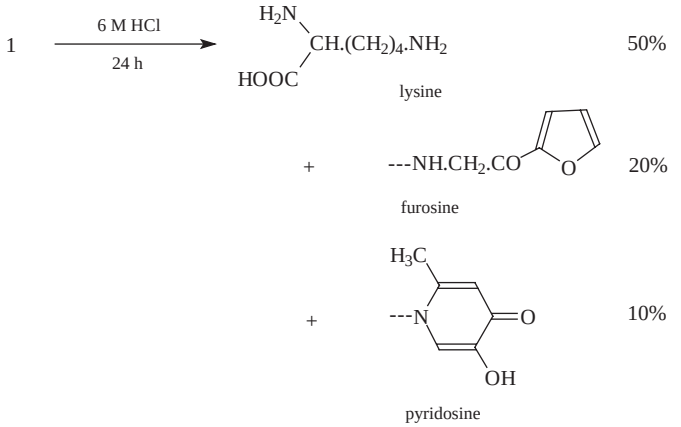
Since lysine is an essential amino acid, its ε-amino group has special significance. The reaction with glucose gives ε-*N*-deoxyfructosyllysine (see Scheme 2.4).

Because of the partial recovery of lysine, the interpretation of the results of the analysis is complicated. It is important to bear in mind that lysine locked up in the Amadori compound, although partially recoverable by amino acid analysis, is no longer nutritionally available.

When lactose reacts with protein in dairy products, lactulosyllysine residues are formed. On hydrolysis, these give yields of 40% recovered lysine and 32% furosine. The lysine residues nutritionally blocked can then be calculated as follows:

$$\%\ \text{Lys nutritionally blocked} = \frac{3.1\ \text{furosine}}{\text{total-Lys} + 1.87\ \text{furosine}} \times 100$$

The factors are derived as follows: 100/32 = 3.1 and 60/32 = 1.87. Total-Lys represents the total lysine recovered in the analysis, *i.e.*, unreacted lysine plus lysine recovered from lactulosyllysine residues. The results obtained are presented in Table 2.1.



Scheme 2.4 *The products of hydrolysis of ε-N-deoxyfructosyllysine under protein-analysis conditions*

Table 2.1 *Lysine damage in good manufacturing practice*[7,31]

	%
Raw or freeze-dried milk	0
Pasteurised (74 °C, 40 s)	0–2
HTST pasteurised (135–150 °C, a few seconds)	0–3
HTST sterilized	5–10
UHT	0–2
Spray-dried powder	0–3
Sweetened condensed	0–3
Sterilised fluid	8–15
Roller-dried (without precondensation)	10–15
Evaporated	15–20
Roller-dried (conventional)	20–50

The requirement for lysine is high in infants (103 as compared with 12 mg kg^{-1} d^{-1} for adults) and, consequently, formula is recommended to contain not less than 6.7 g per 100 g protein. Cow's milk fortunately contains at least 20% more lysine than human milk,[31] but, in any case, roller drying is no longer used in Western countries to produce formula.

That the available lysine in milk-based infant formulae falls on storage and that the furosine obtained increases has been confirmed by Ferrer *et al*.[32] They showed that for an adapted infant formula, the former decreased from 9.78 to 7.85 and 7.45 g kg^{-1} sample in 24 months at 20 and 37 °C, respectively. Furosine increased correspondingly from 187 to 750 and 1001 μg kg^{-1} formula, respectively. For a follow-up infant formula, the corresponding figures were: available lysine from 12.63 to 6.62 and 6.48 g kg^{-1} and furosine from 225 to 758 and 1121 μg kg^{-1}, respectively. It appeared that the furosine increase correlated well with lysine loss over the first 12 months of storage, but less well thereafter.

Henle *et al.*[33] have developed an alternative method for assessing the degree of lysine modification. It is based on complete enzymic hydrolysis by means of pepsin, followed by pronase E and then by aminopeptidase M and prolidase, the resultant lactulosyllysine being determined by ion-exchange chromatography. Results with skim milk and skim milk powder, exposed to different degrees of heat, showed levels of modified lysine 2.5–3.6 times as high as indicated by furosine determination. For infant formula from a range of manufacturers, the ratios were 3.2–5.6. These results have serious, even legal, implications, which need to be addressed.

The kinetics of formation for furosine, as well as those for HMF and lactulose, was investigated as time–temperature integrators for thermal processing of milk in relation to its fat content.[34] From previous experiments, under isothermal and non-isothermal conditions, formation of the three compounds could be described as following pseudo-zero-order kinetics. Knowing the kinetic model allowed the experimental design to be simplified. The kinetics of formation of HMF and lactulose was found not to be affected by fat content; however, for furosine, significant differences were observed between the kinetic parameters for whole, semi-skimmed, and skimmed milk. Nevertheless, these differences were negligible in the context of process impact.

Furosine has also been explored as a quality indicator for foods other than dairy products.[35] Jams gave values of 72.6–629.3 mg per 100 g protein, but reduced-sugar jams (40–55% sugar, rather than 60% or more) gave lower values, 15.1–335.4 mg per 100 g protein. Higher values may thus be due to extra sugar, but also due to lower a_w, higher pH, and more severe processing. Fruit-based infant foods contained 44.0–178 mg per 100 g protein, the presence of citrus juice leading to the highest values.

Earlier, Sanz *et al.*[36] had found tomato pulp to give a little furosine (7.3 mg per 100 g), with only a trace of 2-furoylmethyl-γ-aminobutyric acid, but, on storage for 4 d at 50 °C and an a_w of 0.44, the corresponding amounts were 70.9 and 245.4 mg per 100 g and the amounts for the 2-furoylmethyl derivatives of Ala, Ser + Thr + Glu, and Asp + Asn had come up from 0 to 60.9, 103.5, and 300.5 mg per 100 g, respectively. 2-Furoylmethyl-γ-aminobutyric acid was also the most abundant furoylmethyl derivative obtained from commercial tomato products, ranging from 0 for whole peeled tomatoes to 87.6 mg per 100 g dry matter for double-concentrated tomato paste. Furosine was usually the second most abundant derivative obtained, ranging up to 42.8 mg per 100 g, but its amount exceeded that for 2-furoylmethyl-γ-aminobutyric acid in the two samples of tomato pulp analysed.

Furosine content can also serve as an indicator of the freshness of hen's eggs.[37] It is present in the albumen of fresh eggs at about 10 mg per 100 mg protein, but increases on storage, depending on the temperature, 40 d at 20 °C leading to a value 10 times as high, whereas the concentration in the yolk hardly changes. Maxima of 60 and 90 mg per 100 mg protein in albumen were suggested for EC Grade A-extra and Grade A eggs, respectively. Whole egg was surprisingly found to contain less furosine than expected on the basis of the separate results obtained for albumen and yolk.

Furosine did not prove very useful for the evaluation of dried pasta,[38] but 2-acetyl-3-D-glucopyranosylfuran ranged from < 1 with spaghetti dried under mild conditions to up to 20 ppm with some commercial samples.